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(54) Title: INHIBITORS OF COPPER-CONTAINING AMINE OXIDASES

(57) Abstract: The present invention is directed to 1,3,4-oxadiazine compounds that function as inhibitors of copper-containg amine oxidases commonly known as semicarbazide-sensitive amine oxidases (SSAO), including the human SSAO known as Vascular Adhesion Protein-1 (VAP-1). These SSAO inhibitors have therapeutic utility as drugs to treat conditions and diseases including, but not limited to, a number of inflammatory conditions and diseases (in particular chronic inflammatory conditions such as chronic arthritis, inflammatory bowel diseases, and chronic skin dermatoses), diseases related to carbohydrate metabolism and to abberations in adipocyte differentiation or function and smooth muscle cell function, and vascular diseases. The compounds have the general formula (I): or a tautomer, isomer, hydrazino alcohol degradation product, or a pharmaceutically acceptable solvate, hydrate, or salt thereof, wherein R^1, R^2, R^3, R^4, R^5,R^6, R^7, and R^8 are as defined her ein.

Inhibitors Of Copper-Containing Amine Oxidases

Field of the Invention

The present invention is in the field of medicinal chemistry and is directed to 1,3,4-oxadiazine compounds and their use as inhibitors of copper-containing amine oxidases (E.C. 1.4.3.6) and enzymes of significant identity thereto. The compounds of the present invention have therapeutic utility as drugs to treat diseases including but not limited to a number of inflammatory conditions and diseases (in particular chronic inflammatory conditions or diseases such as chronic arthritis, inflammatory bowel diseases, and chronic skin dermatoses) as well as diseases related to carbohydrate metabolism and to aberrations in adipocyte differentiation or function and smooth muscle cell function.

Background of the Invention

VAP-1 is a human endothelial cell adhesion molecule that has several unique properties that distinguish it from the other inflammation-related adhesion molecules and these are described as follows. VAP-1 has a unique and restricted expression pattern and mediates lymphocyte binding to vascular endothelium (Salmi, M., and Jalkanen, S., *Science 257*:1407-1409 (1992)). Inflammation induces upregulation of VAP-1 to the surface of vascular endothelial cells mediating leukocyte entry to skin, gut and inflamed synovium (Salmi, M., and Jalkanen, S., *Science 257*:1407-1409 (1992); Salmi, M., *et al., J. Exp. Med 178*:2255-2260 (1993); Arvillomi, A., *et al., Eur. J. Immunol. 26*:825-833 (1996); Salmi, M., *et al., J. Clin. Invest. 99*:2165-2172 (1997)). VAP-1 is rapidly translocated onto vascular endothelium at sites of inflammation. (Salmi, M., and Jalkanen, S., *J. Exp. Med. 183*:569-579 (1996); *J. Exp. Med. 186*:589-600 (1997)). Lastly, VAP-1 has a catalytic extracellular domain with a monoamine oxidase activity (Smith, D.J., *et al., J. Exp. Med. 188*:17-27 (1998)).

The cloning and sequencing of the human VAP-1 cDNA revealed that it encodes a transmembrane protein with homology to a class of enzymes called the copper-containing amine oxidases (E.C. 1.4.3.6). Enzyme assays have shown that VAP-1 possesses a monoamine oxidase (MAO) activity which is present in the extracellular domain of the protein (Smith, D.J., *et al., J. Exp. Med. 188*:17-27 (1998)). Thus, VAP-1 is an ecto-enzyme. Analysis of the VAP-1 MAO activity showed that VAP-1 belongs to the class of membrane-bound MAO's termed semicarbazide-sensitive amine oxidases (SSAO). These are distinguished from the widely distributed mitochondrial MAO-A and B flavoproteins by amino acid sequence, cofactor, substrate specificity and sensitivity to certain inhibitors. However, certain substrates and inhibitors are common to both SSAO and MAO activities. The mammalian SSAO's can metabolize various monoamines produced endogenously or absorbed as dietary or xenobiotic substances. They act principally on primary aliphatic or aromatic monoamines such as methylamine or benzylamine (Lyles, G.A., *Int. J. Biochem. Cell Biol. 28*:259-274 (1996)). Thus, VAP-1 located on the vascular endothelial cell surface can act on circulating primary monoamines with the following reaction pathway.

$$RNH_2 + O_2 + H_2O \longrightarrow RCHO + H_2O_2 + NH_3$$

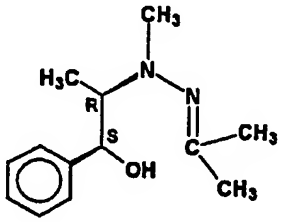
In human clinical tissue samples, expression of VAP-1 is induced at sites of inflammation. This increased level of VAP-1 can lead to increased production of H_2O_2 generated from the action of the VAP-1 SSAO extracellular domain on monoamines present in the blood. This generation of H_2O_2 in the localised environment of the endothelial cell can initiate other cellular events. H_2O_2 is a known signalling molecule that can upregulate other adhesion molecules and this increased adhesion molecule expression may lead to enhanced leukocyte trafficking into areas in which VAP-1 is expressed. Preliminary data supporting this has been obtained using an *in vitro* model in which increased E-Selectin, VCAM-1 and ICAM-1 expression on cultured human umbilical vein endothelial cells (HUVEC) could be observed following addition of purified VAP-1 SSAO protein and benzylamine (an SSAO substrate) to the cell medium. This increase in

adhesion molecule expression was less prominent when mutated (enzymatically inactive) VAP-1 SSAO protein was added instead of the native protein.

A number of 1,3,4-oxadiazines are described in the literature (See, for example, Schmitz, e., *et al.*, *Liebigs Ann. Chem. (6)*:1043-1046 (1983); Samitov, Y.Y., *et al.*, *Zh. Org. Khim. 22(11)*:2271-2277 (1986); Potekhin, A.A., *et al.*, *Khim. Geterotsikl. Soedin. (11)*:1461-1468 (1973); Potekhin, A.A., and Zaitsev, B.D., *Khim. Geterotsikl. Soedin. 7(3)*:301-308 (1971); Ioffe, B.V., and Potekin, A.A., *Tetrahedron Lett. (36)*:3505-3508 (1967); and Kaneko, Japanese Patent Appl. No. 63256951 (1988)). However, use of these compounds as specific SSAO inhibitors apparently has not been disclosed.

In aqueous solution, 1,3,4-oxadiazines may exist in tautomeric hydrazone form. See, for example, Potekhin, A.A., and Zaitsev, B.D., *Khim. Geterotsikl. Soedin. 7(3)*:301-308 (1971), and Ioffe, B.V., and Potekin, A.A., *Tetrahedron Lett. (36)*:3505-3508 (1967).

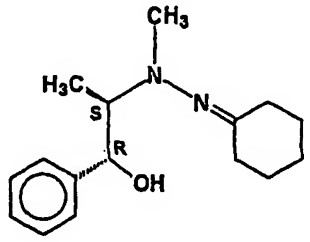
Takahashi, H., *et al.*, *Yakugaku Zasshi 101(12)*:1154-1156 (1981), report the synthesis of a number of *N*-alkylaminoephedrines, including *N*-(isopropylideneamino)-ephedrine (or *R,S*-(+)-(2-hydroxy-1-methyl-2-phenylethyl)methylhydrazone-2-propanone):



These hydrazone compounds were synthesized to evaluate their effect on the bronchial musculature and were found not to exhibit any significant activity. No mention of a 1,3,4-oxadiazine corresponding to the reported hydrazone appears in this reference.

Grifantini, M., *et al.*, *Farmaco, Ed. Sci. 23(3)*:197-203 (1968), report the synthesis of several alkyl- and acyl-derivatives of *N*-amino-1-ephedrine and *N*-amino-d-pseudoephedrine having antidepressant and monoamine oxidase

inhibitory properties. Among the compounds disclosed is the hydrazone erythro-(β-hydroxy-α-methylphenethyl)methylhydrazone cyclohexanone, which has the following structure:



The development of specific VAP-1 SSAO inhibitors that modulate VAP-1 activity would be useful for the treatment of chronic inflammatory conditions or diseases such as chronic arthritis, inflammatory bowel diseases, and chronic skin dermatoses, as well as diseases related to carbohydrate metabolism (including diabetes and complications from diabetes), to aberrations in adipocyte differentiation or function and smooth muscle cell function (in particular, atherscleroșis), and to various vascular diseases.

Summary of the Invention

The present invention is broadly directed to the use of 1,3,4-oxadiazine compounds of Formula *I* as inhibitors of the class of copper-containing amine oxidases known as semicarbazide-sensitive amine oxidases (SSAO), including the human SSAO known as Vascular Adhesion Protein-1 (VAP-1). As VAP-1 SSAO inhibitors, compounds of the present invention can function to prevent leukocyte adhesion events mediated through SSAO activity. Compounds of the present invention are therefore useful for treating a number of inflammatory conditions and diseases of connective tissue, skin, and the gastrointestinal, central nervous system, and pulmonary systems, including such conditions as chronic arthritis, inflammatory bowel diseases, and chronic dermatoses. The compounds are also useful for treating diseases related to carbohydrate metabolism (such as diabetes),

In another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent gastrointestinal inflammatory conditions and diseases, in particular those such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome.

In yet another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat central nervous system inflammatory conditions and diseases, including multiple sclerosis, Alzheimer's disease, and ischaemia-reperfusion injury associated with ischemic stroke.

In another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent pulmonary inflammatory conditions and diseases. In particular, the compounds can be used to treat or prevent such conditions or diseases as asthma and adult respiratory distress syndrome.

In another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent chronic inflammatory skin conditions, especially such inflammatory skin conditions as psoriasis, allegic lesions, lichen planus, and pityriasis rosea.

In yet another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent diseases related to carbohydrate metabolism and complications thereof, such as diabetes and complications from diabetes, microvascular and macrovascular diseases such as atherosclerosis, vascular retinopathies, and neuropathies such as polyneuropathy, mononeuropathies, and autonomic neuropathy.

In still another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent diseases related to or caused by aberrations in adipocyte differentiation or function, such as atherosclerosis or obesity.

In another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent diseases related to or caused by aberrations in smooth muscle cell function, such as athersclerosis.

In another embodiment, the 1,3,4-oxadiazine compounds of Formula *I* are used to treat or prevent vascular diseases, such as atheromatous and

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